UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/687,837	10/13/2000	Peter S. Lu	20054-000210US	7707

24353 7590 03/14/2003

BOZICEVIC, FIELD & FRANCIS LLP
200 MIDDLEFIELD RD
SUITE 200
MENLO PARK, CA 94025

EXAMINER
BUNNER, BRIDGET E

ART UNIT	PAPER NUMBER
1647	20

DATE MAILED: 03/14/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Offic Action Summary	Applicati n No.	Applicant(s)	
	09/687,837	LU ET AL.	
	Examiner	Art Unit	
	Bridget E. Bunner	1647	

-- The MAILING DATE of this communication appears n the cover she t with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 February 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-39* is/are pending in the application.

4a) Of the above claim(s) *7,18-31 and 33-39* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6,8-17 and 32* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-39* are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *13 October 2000* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Status of Application, Amendments and/or Claims

The amendment of 09 April 2002 (Paper No. 9) has been entered in full.

Election/Restrictions

Applicant's election with traverse of Group A, claims 1-6, 8-17, and 32, drawn to an isolated CLASP-2 polynucleotide, an expression vector, a host cell system, and a method of producing a CLASP-2 polypeptide in Paper No. 13 (23 August 2002) is acknowledged. The traversal is on the ground(s) that Groups A, F, and G should be rejoined. This is not found persuasive because, as discussed in the Office Action of 17 June 2002 (Paper No. 11) , the polynucleotide of Invention A can be used in materially different processes other than the methods of Groups F and G, such as DNA purification and gene therapy (MPEP § 086.05(h)). Each of groups A, F, and G are unique invention, requiring a unique search of the prior art. Searching all of the inventions in a single patent application would provide an undue search burden on the examiner and the USPTO's resources because of the non-coextensive nature of these searches.

Applicant's election with traverse of the nucleic acid sequence of SEQ ID NO: 1 in Paper No. 13 (23 August 2002) and the amino acid sequence of SEQ ID NO: 2 in Paper No. 17 (10 December 2002) is acknowledged. The traversal is on the ground(s) that SEQ ID NOs: 1, 3, 5, 9 cover isoforms of human CLASP-2 cDNA and that SEQ ID NOs: 2, 4, 6, 10 cover multiple forms of human CLASP-2 polypeptides produced by alternative exon usage. This is not found persuasive because each polynucleotide and polypeptide sequence is a unique sequence requiring a unique search of the prior art. Searching all of the sequences in the instant application would

provide an undue search burden on the examiner and the USPTO's resources because of the non-coextensive nature of these searches. Additionally, since each of the polynucleotide and polypeptide sequences are different from one another, each may have diverse structural and functional features. Certain positions in the sequence are critical to the polypeptide's structure/function relationship, e.g. such as various sites or regions directly involved in binding, activity and in providing the correct three-dimensional spatial orientation of binding and active sites. These or other regions may also be critical determinants of antigenicity. It is noted that Applicant's arguments directed towards the restriction requirement of a PDZ domain species has been found persuasive and the species have been rejoined.

The requirement is still deemed proper and is therefore made FINAL.

Claims 7, 18-31, and 33-39 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected group, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in Paper No. 14 (04 September 2002).

It is noted to Applicant that although claims 30 and 31 may recite unintentional errors, Applicant must comply with 37 CFR 1.121 to make claim amendments.

Claims 1-6, 8-17, and 32 are under consideration in the instant application.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 14 May 2001 (Paper No. 6) was considered by the examiner.

Sequence Compliance

The Applicant's response to the Notice to Comply with Sequence Listing Requirements under 37 CFR §1.821 (Paper No. 9, 09 April 2002) has been considered and is found persuasive. Therefore, the requirements set forth in the Notice to Comply (Paper No. 14, 03 December 2001) are withdrawn.

Specification

1. The disclosure is objected to because of the following informalities:

1a. Patent applications are referenced throughout the disclosure (pg 2, line 10; pg 35, line 23). The status of the applications must be updated.

1b. At page 117, line 5, it is not clear of the meaning of the following sentence: "(Please provide some specific regions)."

1c. The specification at page 118, line 7 contains a reference to a specific product catalog number, which is subject to change over time. (Note, this issue could be overcome by adding the complete catalog information, such as year and volume or by deleting the catalog number.)

1d. The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code (See page 15, lines 19). Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP § 608.01.

1e. The Brief Description of Drawings at pg 10 of the specification does not refer to Figures 10A-10H.

1f. The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed.

The following title is suggested: "NUCLEIC ACID MOLECULE ENCODING A CLASP-2 TRANSMEMBRANE PROTEIN".

Appropriate correction is required.

Claim Objections

2. Claims 1, 4-6, and 14-15 are objected to because of the following informalities:

2a. Claim 1, 4-6, 14-15 recite non-elected groups.

2b. Claim 1(c) at line 3 is missing the term "least" before the phrase "25 contiguous residues".

2c. Claim 4, line 1 requires the insertion of a space keystroke between the word "claim 1" and "that".

Appropriate correction is required.

Double Patenting

3. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 1-6, 8-17, and 32 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-6, 8-17, and 32 of copending Application No. 09/547,276. Although the conflicting claims are not identical, they are not patentably distinct from each other. The claims in the '276 application and the instant application recite an isolated Cadherin-like asymmetry protein-2 (CLASP-2) polynucleotide wherein the polynucleotide is (a) a polynucleotide that has the sequence of SEQ ID NO: 1, (b) a

polynucleotide that hybridizes under stringent hybridization conditions to (a) and encodes a polypeptide having the sequence of SEQ ID NO: 2 or an allelic variant or homologue, (c) a polynucleotide that hybridizes under stringent hybridization conditions to (a) and encodes a polypeptide with at least 25 contiguous residues of the polypeptide of SEQ ID NO: 2, or (d) a polynucleotide that hybridizes under stringent hybridization conditions to (a) and has at least 12 contiguous bases identical to or exactly complementary to SEQ ID NO: 1. Both sets of claims further recite that the polynucleotide encodes a polypeptide having the sequence of SEQ ID NO: 2 that specifically binds to a PDZ domain of DLG1. The claims recite an isolated CLASP-2 polynucleotide comprising a nucleotide sequence that has at least 90% identity to SEQ ID NO: 1. The sets of claims in the pending applications also recite an expression vector comprising the polynucleotide, a host cell, and a method for producing the polypeptide. Additionally, the claims are directed to an antisense oligonucleotide complementary to a mRNA comprising SEQ ID NO: 1 and an antisense polynucleotide less than about 200 bases in length. The claims recite a pharmaceutical composition comprising a polynucleotide and a pharmaceutically acceptable carrier. The difference between the claims of the '276 application and the instant application is the hybridization conditions recited in claim 1(b)-(d). The '276 application recites specification hybridization conditions while the instant application does not.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Claim Rejections - 35 USC § 101 and § 112, first paragraph

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. Claims 1-6, 8-17, and 32 are rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a credible, specific and substantial asserted utility or a well established utility. Novel biological molecules lack well established utility and must undergo extensive experimentation.

Specifically, claims 1-6, 8-17, and 32 are directed to an isolated Cadherin-like asymmetry protein-2 (CLASP-2) polynucleotide wherein the polynucleotide is (a) a polynucleotide that has the sequence of SEQ ID NO: 1, (b) a polynucleotide that hybridizes under stringent hybridization conditions to (a) and encodes a polypeptide having the sequence of SEQ ID NO: 2 or an allelic variant or homologue, (c) a polynucleotide that hybridizes under stringent hybridization conditions to (a) and encodes a polypeptide with at least 25 contiguous residues of the polypeptide of SEQ ID NO: 2, or (d) a polynucleotide that hybridizes under stringent hybridization conditions to (a) and has at least 12 contiguous bases identical to or exactly complementary to SEQ ID NO: 1. The claims further recite that the polynucleotide encodes a polypeptide having the sequence of SEQ ID NO: 2 that specifically binds to a PDZ domain of DLG1. The claims recite an isolated CLASP-2 polynucleotide comprising a nucleotide sequence that has at least 90% identity to SEQ ID NO: 1. The claims also recite an expression vector comprising the polynucleotide, a host cell, and a method for producing the polypeptide. Additionally, the claims are directed to an antisense oligonucleotide complementary to a mRNA

comprising SEQ ID NO: 1 and an antisense polynucleotide less than about 200 bases in length. The claims recite a pharmaceutical composition comprising a polynucleotide and a pharmaceutically acceptable carrier.

The specification asserts that the CLASP-2 polynucleotide (SEQ ID NO:1) and polypeptide (SEQ ID NO: 2) of the present invention are involved in a variety of cellular processes, particularly related to immune function, T cell activation, regulation of T cell and B cell interactions, and in the organization, establishment, and maintenance of the "immunological synapse" (including signal transduction, cytoskeletal interactions, and membrane organization) (pg 20, lines 32-33; pg 21, lines 1-5). However, the instant specification does not teach any significant characteristics of the CLASP-2 polynucleotide (SEQ ID NO: 1) or polypeptide (SEQ ID NO: 2). The specification also does not disclose any methods or working examples that indicate the polynucleotide and polypeptide of the instant invention are involved in any of the abovementioned activities. Since significant further research would be required of the skilled artisan to determine how the claimed polypeptide is involved with the above-mentioned activities, the asserted utilities are not substantial.

Since the utility is not presented in mature form and significant further research is required, the utility is not substantial. The specification asserts the following as patentable utilities for the claimed putative polynucleotide (SEQ ID NO: 1):

1) to detect the expression of CLASP-2 in cells (pg 45, lines 31-32; pg 51-54)

2) in the diagnosis of a disorder or disease resulting from aberrant expression of CLASP-2 (pg 46, lines 1-19)

3) as hybridization probes for cDNA and genomic DNA (pg 46, lines 20-33; pg 47; pg 48, lines 1-25)

4) as primers for a nucleic acid amplification (pg 46, lines 20-33; pg 47; pg 48, lines 1-25)

5) to treat, detect, or modulate immune system disorders, hematopoietic cell disorders, allergic reactions, organ rejection or graft-versus-host disease, inflammation, infectious agents (pg 48, lines 26-34; pg 49-51)

6) to engineer hammerhead motif ribozyme molecules (pg 58, lines 25-34; pg 59, lines 1-4, 30-34)

7) for gene therapy (pg 60-64)

8) to construct a transgenic animal (pg 64-66)

9) in chromosome mapping (pg 66, lines 5-27)

10) to screen CLASP-2 agonists and antagonists (pg 45, lines 26-28)

Each of these shall addressed in turn.

1) to detect the expression of CLASP-2 in cells. This asserted utility is not specific or substantial. The specification does not disclose a specific target sequence. The specification does not disclose the cell types that express CLASP-2. Significant further experimentation would be required of the skilled artisan to identify cells and/or tissues and organs with CLASP-2. Since this asserted utility is also not present in mature form so that it could be readily used in real world sense, the asserted utility is not substantial.

2) in the diagnosis of a disorder or disease resulting from aberrant expression of CLASP-2. This asserted utility is not specific or substantial. The specification does not disclose disorders associated with a mutated, deleted, or translocated CLASP-2 gene. Significant further experimentation would be required of the skilled artisan to identify individuals with such a disease. Since this asserted utility is also not present in mature form so that it could be readily used in real world sense, the asserted utility is not substantial.

3) as hybridization probes for cDNA and genomic DNA. This asserted utility is not substantial or specific. Hybridization probes can be designed from any polynucleotide sequence. Further, the specification does not disclose specific cDNA, DNA, or RNA targets. Since this asserted utility is also not present in mature form, so that it could be readily used in a real world sense, the asserted utility is not substantial.

4) as primers for a nucleic acid amplification. This asserted utility is not substantial or specific. Primers can be designed from any polynucleotide sequence. Further, the specification does not disclose a specific DNA target. Since this asserted utility is also not present in mature form, so that it could be readily used in a real world sense, the asserted utility is not substantial.

5) to treat, detect, or modulate immune system disorders, hematopoietic cell disorders, allergic reactions, organ rejection or graft-versus-host disease, inflammation, infectious agents. This asserted utility is not specific or substantial. The specification does not disclose disorders associated with a mutated, deleted, or translocated CLASP-2 gene (SEQ ID NO: 1). The specification does not disclose which disorders are associated with altered levels of the CLASP-2 gene. Significant further experimentation would be required of the skilled artisan to identify individuals with such a disease. Since this asserted utility is also not present in mature form, so that it could be readily used in a real world sense, the asserted utility is not substantial.

6) to engineer hammerhead motif ribozyme molecules. This asserted utility is not specific or substantial. Ribozymes can be designed from any DNA/RNA sequence. Additionally, the specification does not disclose a specific DNA/RNA target. Since this asserted utility is also not present in mature form, so that it could be readily used in a real world sense, the asserted utility is not substantial.

7) for gene therapy. This asserted utility is not specific or substantial. The specification does not disclose diseases associated with a mutated, deleted, or translocated CLASP-2 gene of SEQ ID NO: 1. Significant further experimentation would be required of the skilled artisan to identify individuals with such a disease. Since this asserted utility is also not presented in mature form, so that it could be readily used in a real world sense, the asserted utility is not substantial.

8) to construct a transgenic animal. This asserted utility is not specific or substantial. The specification does not disclose diseases associated with a mutated, deleted, or translocated CLASP-2 gene (SEQ ID NO: 1). Significant further experimentation would be required of the skilled artisan to identify such a disease. The specification discloses nothing about whether the gene will be "knocked in" or "knocked out" or what specific tissues and cells are being targeted. Since this asserted utility is also not present in mature form, so that it could be readily used in a real world sense, the asserted utility is not substantial.

9) in chromosome mapping. This asserted utility is not substantial or specific. Such assays can be performed with any polynucleotide. Further, the specification does not disclose a specific DNA target. Since this asserted utility is also not present in mature form, so that it could be readily used in a real world sense, the asserted utility is not substantial.

10) to screen CLASP-2 agonists and antagonists. This asserted utility is not specific or substantial. Such assays can be performed with any polynucleotide. Nothing is disclosed about how the polynucleotide is affected by the compounds. Additionally, the specification discloses nothing specific or substantial for the CLASP-2 agonists and antagonists screened in this method. Since this asserted utility is also not presented in mature form, so that it could be readily used in a real world sense, the asserted utility is not substantial.

The specification of the instant application also teaches that the CLASP-2 C-terminal 20 amino acids bind to PDZ domain-containing proteins (pg 9-10, 28-29, 120-121; Figure 9). However, relevant literature teaches that PDZ domains are among the commonest protein domains represented in sequence genomes and analysis of the human genome estimates the presence of 440 PDZ domains in at least 259 different proteins (Hung et al. J Biol Chem 277(8): 5699-5702, 2002; ¶ 1). Therefore, the asserted utility of CLASP-2 or CLASP-2 related compounds to bind to PDZ-domain containing proteins and to mediate changes in multiple protein-protein interactions involved in the function of lymphoid tissues is not specific or substantial (specification, pg 29, lines 1-6). At least 258 other PDZ-domain containing proteins could bind to CLASP-2 and/or the PDZ-domain containing proteins used in the experiments of the instant application. The specification also does not disclose any *specific* proteins that CLASP-2 may interact with in lymphoid tissue or during cell signaling. Since this asserted utility is also not presented in mature form, so that it could be readily used in a real world sense, the asserted utility is not substantial.

Additionally, the utilization of CLASP-2 expression to determine T-cell activation is not a specific and substantial utility. Although Applicant indicates that CLASP-2 expression levels decrease at 1 hour, 2 hours, and 4 hours after activation (pg 123, lines 10-20; Figure 14), it cannot be determined if this decrease is a significant difference as compared to T-cells that have not been activated. If the decrease in CLASP-2 expression is not significant between the two cell types, then this utility is not specific because the skilled artisan would not be able to distinguish activated T-cells from inactivated T-cells. Since this asserted utility is also not presented in mature form, so that it could be readily used in a real world sense, the asserted

utility is not substantial. Applicant is encouraged to submit any evidence under 37 C.F.R. 1.132 that would indicate a significant difference between the expression of CLASP-2 in activated and inactivated T-cells.

4. Claims 1-6, 8-17, and 32 are also rejected under 35 U.S.C. 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention.

Claims 1-6, 8-17, and 32 recite a CLASP-2 polynucleotide wherein the polynucleotide is a polynucleotide that hybridizes under stringent hybridization conditions to (a) and encodes a polypeptide having the sequence of SEQ ID NO: 2 or an allelic variant or homologue, a polynucleotide that hybridizes under stringent hybridization conditions to (a) and encodes a polypeptide with at least 25 contiguous residues of the polypeptide of SEQ ID NO: 2, or (d) a polynucleotide that hybridizes under stringent hybridization conditions to (a) and has at least 12 contiguous bases identical to or exactly complementary to SEQ ID NO: 1. The claims also recite an isolated CLASP-2 polynucleotide comprising a nucleotide sequence that has at least 90% identity to SEQ ID NO: 1. Additionally, the claims are directed to an antisense polynucleotide less than about 200 bases in length.

The specification discloses that "the CLASP-2 variants of the invention can contain alterations in the coding regions, non-coding regions, or both" (pg 42, lines 12-13). The specification teaches that known methods of protein engineering and recombinant DNA technology can generate variants to improve or alter the characteristics of the CLASP-2

polypeptides (pg 44, lines 4-33). However, the specification does not teach any allelic variants or homologs of the CLASP-2 polynucleotide or polypeptide. The specification does not disclose (i) a polynucleotide that encodes a polypeptide with at least 25 contiguous residues of the polypeptide of SEQ ID NO: 2, (ii) a polynucleotide that has at least 12 contiguous bases identical to or exactly complementary to SEQ ID NO: 1, or (iii) an antisense polynucleotide less than about 200 bases in length. The specification also does not teach a nucleic acid sequence with 90% sequence identity to the nucleotide sequence of SEQ ID NO: 1. Furthermore, the specification does not disclose any chromosomal locus for CLASP-2, which is a necessity since an allelic variant must be at the same locus. Undue experimentation is required to identify the locus and map variants to determine which ones are alleles. Additionally, the specification does not teach functional or structural characteristics of any polynucleotide variants in the context of a cell or organism.

Generally, the art acknowledges that function cannot be predicted based solely on structural similarity to a protein found in the sequence databases. For example, Skolnick et al. (2000, Trends in Biotech. 18:34-39) state that knowing the protein structure by itself is insufficient to annotate a number of functional classes, and is also insufficient for annotating the specific details of protein function (see Box 2, p. 36). Similarly, Bork (2000, Genome Research 10:398-400) states that the error rate of functional annotations in the sequence database is considerable, making it even more difficult to infer correct function from a structural comparison of a new sequence with a sequence database (see especially p. 399). Such concerns are also echoed by Doerks et al. (1998, Trends in Genetics 14:248-250) who state that (1) functional information is only partially annotated in the database, ignoring multi functionality, resulting in

underpredictions of functionality of a new protein and (2) overpredictions of functionality occur because structural similarity often does not necessarily coincide with functional similarity. Smith et al. (1997, Nature Biotechnology 15:1222-1223) remark that there are numerous cases in which proteins having very different functions share structural similarity due to evolution from a common ancestral gene. Brenner (1999, Trends in Genetics 15:132-133) argues that accurate inference of function from homology must be a difficult problem since, assuming there are only about 1000 major gene superfamilies in nature, then most homologs must have different molecular and cellular functions. Finally, Bork et al. (1996, Trends in Genetics 12:425-427) add that the software robots that assign functions to new proteins often assign a function to a whole new protein based on structural similarity of a small domain of the new protein to a small domain of a known protein. Such questionable interpretations are written into the sequence database and are then considered facts.

Therefore, based on the discussions above concerning the specific examples of structurally similar proteins that have different functions, along with the art's recognition that one cannot rely upon structural similarity alone to determine functionality, the specification fails to teach the skilled artisan how to use the claimed polynucleotide to make biologically active CLASP-2 without resorting to undue experimentation to determine what the specific biological activities of the polypeptide are.

The specification does not teach the skilled artisan how to use the claimed polynucleotides encoding CLASP-2 for purposes unrelated to the asserted biological activity. For example, there is no disclosure of particular disease states correlating to an alteration in levels or forms of the polypeptide such that the claimed polynucleotide encoding CLASP-2

could be used as a diagnostic tool. Therefore, the skilled artisan is not provided with sufficient guidance to use the claimed polynucleotides for any purpose.

Further, the problem of predicting protein and DNA structure from sequence data and in turn utilizing predicted structural determinations to ascertain functional aspects of the protein and DNA is extremely complex. For example, while it is known that many amino acid substitutions are generally possible in any given protein the positions within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation of success are limited. Certain positions in the sequence are critical to the protein's structure/function relationship, e.g. such as various sites or regions directly involved in binding, activity and in providing the correct three-dimensional spatial orientation of binding and active sites. These or other regions may also be critical determinants of antigenicity. These regions can tolerate only relatively conservative substitutions or no substitutions (see Wells, 1990, Biochemistry 29:8509-8517; Ngo et al., 1994, The Protein Folding Problem and Tertiary Structure Prediction, pp. 492-495). However, Applicant has provided little or no guidance beyond the mere presentation of sequence data to enable one of ordinary skill in the art to determine, without undue experimentation, the positions in the protein and DNA which are tolerant to change and the nature and extent of changes that can be made in these positions. The art recognizes that function cannot be predicted from structure alone (Bork, 2000, Genome Research 10:398-400; Skolnick et al., 2000, Trends in Biotech. 18(1):34-39, especially p. 36 at Box 2; Doerks et al., 1998, Trends in Genetics 14:248-250; Smith et al., 1997, Nature Biotechnology 15:1222-1223; Brenner, 1999, Trends in Genetics 15:132-133; Bork et al., 1996, Trends in Genetics 12:425-427).

Furthermore, claims 13-14 and 32 are directed to an antisense polynucleotide/oligonucleotide and a pharmaceutical composition comprising a CLASP-2 polynucleotide and a pharmaceutically acceptable carrier. The specification teaches a composition comprising an isolated CLASP-2 polynucleotide consisting of the nucleic acid sequence of SEQ ID NO: 1. The specification does not teach how to use an antisense polynucleotide/oligonucleotide or CLASP-2 "pharmaceutical" composition and a "pharmaceutically acceptable carrier" without undue experimentation for the treatment of a disease in an animal. The specification lists disorders to be treated (pg 49-54), but there are no working examples directed to a particular disorder in an animal or administration of an antisense polynucleotide/oligonucleotide or CLASP-2 polynucleotide consisting of the nucleic acid sequence of SEQ ID NO: 1 to an animal for treatment. (Note, part of this issue could be overcome by deleting the terms "pharmaceutical" and "pharmaceutically acceptable" from the claims.)

Due to the large quantity of experimentation necessary to determine an activity or property of the disclosed polypeptide such that it can be determined how to use the claimed polynucleotides encoding CLASP-2, to generate the infinite number of derivatives recited in the claims and possibly screen same for activity, and to determine the quantity of CLASP-2 polynucleotide to be administered, the most effective administration route, and the duration of the treatment, the lack of direction/guidance presented in the specification regarding same, the absence of working examples directed to same, the complex nature of the invention, the state of the prior art establishing that biological activity cannot be predicted based on structural similarity and the unpredictability of the effects of mutation on protein structure and function, the

unpredictability of the effects of the CLASP-2 polynucleotide *in vivo,* and the breadth of the claims which fail to recite particular biological activities and also embrace a broad class of structural fragments and variants, undue experimentation would be required of the skilled artisan to make and/or use the claimed invention in its full scope.

6. Claims 1-6, 8-17, and 32 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Specifically, claims 1-6, 8-17, and 32 are directed a CLASP-2 polynucleotide wherein the polynucleotide is a polynucleotide that hybridizes under stringent hybridization conditions to (a) and encodes a polypeptide having the sequence of SEQ ID NO: 2 or an allelic variant or homologue, a polynucleotide that hybridizes under stringent hybridization conditions to (a) and encodes a polypeptide with at least 25 contiguous residues of the polypeptide of SEQ ID NO: 2, or (d) a polynucleotide that hybridizes under stringent hybridization conditions to (a) and has at least 12 contiguous bases identical to or exactly complementary to SEQ ID NO: 1. The claims also recite an isolated CLASP-2 polynucleotide comprising a nucleotide sequence that has at least 90% identity to SEQ ID NO: 1. Additionally, the claims are directed to an antisense polynucleotide less than about 200 bases in length.

The specification teaches human a CLASP-2 polynucleotide and polypeptide (SEQ ID NO: 1 and SEQ ID NO: 2, respectively). The specification also discloses that "the CLASP-2 variants of the invention can contain alterations in the coding regions, non-coding regions, or

both" (pg 42, lines 12-13). The specification teaches that known methods of protein engineering and recombinant DNA technology can generate variants to improve or alter the characteristics of the CLASP-2 polypeptides (pg 44, lines 4-33). However, the specification does not teach functional or structural characteristics of the polynucleotide variants in the context of a cell or organism. The description of one CLASP-2 polynucleotide species (SEQ ID NO: 1) and one CLASP-2 polypeptide species (SEQ ID NO: 2) is not adequate written description of an entire genus of functionally equivalent polynucleotides and polypeptides which incorporate all variants and fragments (i) with at least 90% sequence identity to the human CLASP-2 polynucleotide comprising SEQ ID NO: 1. The description of one CLASP-2 polynucleotide species and one CLASP-2 polypeptide species is also not adequate written description of an entire genus of functionally equivalent polynucleotides and polypeptides which incorporate all variants and fragments (ii) that encode a polypeptide with at least 25 contiguous residues, (iii) have at least 12 bases identical to or exactly complementary to SEQ ID NO: 1, and (iv) have antisense polynucleotide less than 200 bases in length.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed"* (See page 1117). The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed" (See *Vas-Cath* at page 1116).

With the exception of the sequences referred to above, the skilled artisan cannot envision the detailed chemical structure of the encompassed polynucleotides, and therefore conception is

not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. The nucleic acid itself is required. See *Fiers v. Revel*, 25 USPQ2d 1601 at 1606 (CAFC 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016.

One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481 at 1483. In *Fiddes*, claims directed to mammalian FGF's were found to be unpatentable due to lack of written description for that broad class. The specification provided only the bovine sequence.

Therefore, only an isolated CLASP-2 polynucleotide that has the sequence of SEQ ID NO: 1, but not the full breadth of the claim meets the written description provision of 35 U.S.C. §112, first paragraph. Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

7. Claim 5 is rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The invention appears to employ novel nucleic acid molecules (i.e., clones AVC-PD1, AVC-PD2). Since the nucleic acid molecules are essential to the claimed invention they must be obtainable by a repeatable method set forth in the specification or otherwise readily available to the public. If the nucleic acid molecules are not so obtainable or available, the

requirements of 35 U.S.C. § 112 may be satisfied by a deposit of the nucleic acid molecules. The specification does not disclose a repeatable process to obtain the nucleic acid molecules and it is not apparent if the nucleic acid molecules are readily available to the public. It is noted that Applicant has deposited the nucleic acid molecules (p. 109 of the specification), but there is no indication in the specification as to public availability. If the deposit is made under the Budapest Treaty, then an affidavit or declaration by Applicant, or a statement by an attorney of record over his or her signature and registration number, stating that the specific nucleic acid molecules have been deposited under the Budapest Treaty and that the nucleic acid molecules will be irrevocably and without restriction or condition released to the public upon the issuance of a patent, would satisfy the deposit requirement made herein. If the deposit has not been made under the Budapest Treaty, then in order to certify that the deposit meets the criteria set forth in 37 C.F.R. §§ 1.801-1.809, Applicant may provide assurance of compliance by an affidavit or declaration, or by a statement by an attorney of record over his or her signature and registration number, showing that:

(a) during the pendency of this application, access to the invention will be afforded to the Commissioner upon request;
(b) all restrictions upon availability to the public will be irrevocably removed upon granting of the patent;

(c) the deposit will be maintained in a public depository for a period of 30 years or 5 years after the last request or for the effective life of the patent, whichever is longer;

(d) a test of the viability of the biological material at the time of deposit will be made (see 37 C.F.R. § 1.807); and

(e) the deposit will be replaced if it should ever become inviable.

Applicant's attention is directed to M.P.E.P. §2400 in general, and specifically to §2411.05, as well as to 37 C.F.R. § 1.809(d), wherein it is set forth that "the specification shall

contain the accession number for the deposit, the date of the deposit, the name and address of the depository, and a description of the deposited material sufficient to specifically identify it and to permit examination." The specification should be amended to include such, however, Applicant is cautioned to avoid the entry of new matter into the specification by adding any other information.

35 USC § 112, second paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

8. Claims 1-6, 8-17, and 32 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

9. Regarding claims 2-3, the acronyms "PDZ", "PSD95", "DLG1", and "neDLG" render the claims vague and indefinite. Abbreviations should be spelled out in all independent claims for clarity.

10. Stringency is relative, and the art does not recognize a single set of conditions as stringent. The specification also does not provide an unambiguous definition for the term. In the absence of a recitation of clear hybridization conditions (e.g., "hybridizes at wash conditions of **A** X SSC and **B** % SDS at **C**°C"), claims 1-6, 8-17, and 32 fail to define the metes and bounds of the varying structures of polynucleotides recited in the claimed methods.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

10. Claims 1, 8-13, 17, and 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pianese et al. (Genbank Accession No. X68101; 24 February 1999) in view of Sibson et al. (WO 94/01548).

Pianese et al. teach an isolated polynucleotide that encodes a polypeptide with at least 25 contiguous residues of the polypeptide of SEQ ID NO: 2 of the instant application (See sequence alignment attached to this Office Action as Appendix A; see nucleotides 365-700 of Pianese et al.; see also amino acids 864-975 of SEQ ID NO: 2 of the instant application). Pianese et al. also teach a polynucleotide that has at least 12 contiguous bases identical to or exactly complementary to SEQ ID NO: 1 of the instant application and an antisense polynucleotide less than 200 bases in length (See sequence alignment attached to this Office Action as Appendix B; see nucleotides 230-270 of Pianese et al.; see nucleotides 2466-2496 of SEQ ID NO: 1 of the instant application, for example).

Pianese et al. does not teach expression vectors, host cells, or a method of producing a polypeptide.

Sibson et al. discloses that it is generally useful to place a desired cDNA sequence into an expression vector, host cell, and express the encoded protein (see pages 8-13).

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to use Pianese's DNA and the expression vector, host cell, and method of expressing and then isolating the encoded polypeptide as taught by Sibson et al. in view of Sibson's suggestion that it would be desirable to do so, as cited above.

Conclusion

No claims are allowable.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure:

NCI-CGAP. Accession No. AA484945, EST database, 15 August 1997.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Bridget E. Bunner whose telephone number is (703) 305-7148. The examiner can normally be reached on 8:30-5:30 M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Kunz can be reached on (703) 308-4623. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 872-9306 for regular communications and (703) 872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 872-9305.

Elizabeth C. Kemmerer

BEB
Art Unit 1647
February 27, 2003

...GAGCAACACATTACATGTA...

seq_name: gb_ro:RNTRG

seq_documentation_block:

```
LOCUS       RNTRG                  3227 bp    mRNA    linear   ROD 24-FEB-1999
DEFINITION  R.norvegicus trg mRNA.
ACCESSION   X68101
VERSION     X68101.1  GI:550419
KEYWORDS    trg gene.
SOURCE      Norway rat.
  ORGANISM  Rattus norvegicus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae;
            Rattus.
REFERENCE   1  (bases 1 to 3227)
  AUTHORS   Pianese,L.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-AUG-1992) L. Pianese, Dipartimento di Biologia,
            Patologia Cellulare e Molecolare, Via Pansini, Naples, ITALY
REFERENCE   2  (bases 1 to 3227)
  AUTHORS   Pianese,L., Porcellini,A., Avvedimento,V.E., D'Esposti,F.,
            Feliciello,A., Monticelli,A., Musti,A.M., Tortora,G., Varrone,S.
            and Cocozza,S.
  TITLE     A novel thyroid transcript negatively regulated by tsh
  JOURNAL   Mol. Biol. 13, 75-83 (1994)
FEATURES             Location/Qualifiers
     source          1. .3227
                     /organism="Rattus norvegicus"
                     /strain="Fischer"
                     /db_xref="taxon:10116"
                     /cell_line="FRTL-"
                     /clone_lib="lambdaGT"
     gene            1. .2218
                     /gene="trg"
     CDS             <1. .2218
                     /gene="trg"
                     /codon_start=2
                     /protein_id="CAA48220.1"
                     /db_xref="GI:550420"
                     /db_xref="SPTREMBL:Q63603"
                     /translation="KLSRGHSPLMKKVFDVYLCFLQKHQSEMALKNVFTALRSLIYKF
                     PSTFYEGRADMCASLCYEVLKCCNSKLSSIRTEASQLLYFLMRNNFDYTGKKSFVRTH
                     LQVIISLSQLIADVVGIGGTRFQQSLSIINNCANSDRLIKHTSFSSDVKDLTKRIRTV
                     LMATAQMKEHENDPEMLVDLQYSLAKSYASTPELRKTWLDSMARIHVKNGDLSEAAMC
                     YVHVTALVAEYLTRKEADLALQREPPVFPYSHTSCQRKSRGGMFRQGCTAFRVITPNI
                     DEEASMMEDVGMQDVHFNEDVLMELLEQCADGLWKAERLRAGLLTSINSSSPSMKSGG
                     TLETTHLYDTLHRPYSKVTEVITRAAGSWDLLPGGLFGQGFFEDEDGKEYIYKEPKLT
                     PLSEISQRLLKLYSDKFGSENVKMIQDSGKVNPKDLDSKFAYIQVTHVTPFFDEKELQ
                     ERKTEFERCHNIRRFMFEMPFTQTGKRQGGVEEQCKRRTILTAIHCFPYVKKRIPVMY
                     QHHTDLNPIEVAIDEMSKKVAELHQLCSSAEVDMIKLQLKLQGSVSVQVNAGPLAYAR
                     AFLDDTNTKRYPDNKVKLLKEVFRQFVEACGQALAVNERLIKEDQLEYQEEMKANYRE
                     IRKELSDIIVPRICPGEDKRATKFPAHLQRHQRDTNKHSGSRVDQFILSCVTLPHEPH
                     VGTCFVMCKLRTTFRANHWFCQAQEEAMGNGREKEPWTVIFNSRFYRSWGKVHIFF"
BASE COUNT      905 a    738 c    777 g    807 t
ORIGIN
```

alignment_scores:

```
            Quality: 2882.00              Length:    674
              Ratio:  4.827                Gaps:      6
 Percent Similarity:  88.576   Percent Identity: 86.202
```

alignment_block:

US-09-547-276-2 x RNTRG ..

Align seg 1/1 to: RNTRG from: 1 to: 3227

```
    747 GlyHisAsnProLeuMetLysLysValPheAspValTyrLeuCysPheLe 763
        |||||||:::||||||||||||||||||||||||||||||||||||||||
     14 GGACATAGTCCACTCATGAAGAAAGTTTTTGATGTCTACCTGTGTTTCCT  63

    763 uGlnLysHisGlnSerGluThrAlaLeuLysAsnValPheThrAlaLeuA 780
        ||||||||||||||||||||   ||||||||||||||||||||||||||||
     64 CCAAAAGCATCAGTCAGAAATGGCTTTAAAAAATGTCTTCACTGCCTTAA 113

    780 rgSerLeuIleTyrLysPheProSerThrPheTyrGluGlyArgAlaAsp 796
        ||||||||||||||||||||||||||||||||||||||||||||||||||
    114 GGTCATTAATTTATAAGTTCCCCTCAACGTTCTACGAGGGGCGGGCAGAC 163

    797 MetCysAlaAlaLeuCysTyrGluIleLeuLysCysCysAsnSerLysLe 813
        |||||||||:::|||||||||||||:::|||||||||||||||||||||
    164 ATGTGCGCATCTCTGTGCTATGAGGTTCTCAAGTGCTGTAACTCCAAGCT 213

    813 uSerSerIleArgThrGluAlaSerGlnLeuLeuTyrPheLeuMetArgA 830
        ||||||||||||||||||||||||||||||||||||||||||||||||||
    214 CAGCTCCATCCGCACCGAGGCCTCCCAGCTGCTCTACTTCCTGATGAGGA 263

    830 snAsnPheAspTyrThrGlyLysLysSerPheValArgThrHisLeuGln 846
        ||||||||||||||||||||||||||||||||||||||||||||||||||
    264 ACAACTTCGACTACACAGGGAAGAAGTCTTTTGTCCGGACACACTTACAG 313

    847 ValIleIleSerValSerGlnLeuIleAlaAspValValGlyIleGlyGl 863
        ||||||||||||:::||||||||||||||||||||||||||||||||||
    314 GTCATCATCTCTCTCAGCCAGCTGATCGCAGACGTGGTTGGCATTGGAGG 363

    863 uThrArgPheGlnGlnSerLeuSerIleIleAsnAsnCysAlaAsnSerA 880
        ||||||||||||||||||||||||||||||||||||||||||||||||||
    364 AACCAGATTCCAGCAGTCCTTGTCTATCATCAACAACTGTGCCAATAGCG 413

    880 spArgLeuIleLysHisThrSerPheSerSerAspValLysAspLeuThr 896
        ||||||||||||||||||||||||||||||||||||||||||||||||||
    414 ACCGGCTCATTAAGCACACCAGCTTCTCCTCTGATGTGAAGGATTTGACC 463

    897 LysArgIleArgThrValLeuMetAlaThrAlaGlnMetLysGluHisGl 913
        ||||||||||||||||||||||||||||||||||||||||||||||||||
    464 AAGAGGATCCGCACAGTCCTGATGGCCACAGCCCAGATGAAGGAACATGA 513

    913 uAsnAspProGluMetLeuValAspLeuGlnTyrSerLeuAlaLysSerT 930
        ||||||||||||||||||||||||||||||||||||||||||||||||||
    514 GAACGACCCAGAGATGCTGGTGGACCTGCAGTACAGCCTGGCCAAGTCCT 563

    930 yrAlaSerThrProGluLeuArgLysThrTrpLeuAspSerMetAlaArg 946
        ||||||||||||||||||||||||||||||||||||||||||||||||||
    564 ACGCCAGCACCCCTGAGCTCAGGAAGACATGGCTGGACAGCATGGCAAGG 613
```

Appendix A (cont.)

```
 947 IleHisValLysAsnGlyAspLeuSerGluAlaAlaMetCysTyrValHi 963
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 614 ATCCATGTTAAAAATGGGGATCTCTCAGAGGCAGCAATGTGCTATGTCCA 663

 963 sValThrAlaLeuValAlaGluTyrLeuThrArgLys..............  975
     ||||||||||||||||||||||||||||||||||||
 664 CGTGACAGCTTTGGTAGCGGAATATCTCACACGGAAAGAAGCTGACCTAG 713

 975 ..................................................  975

 714 CACTCCAGCGGGAACCACCTGTCTTCCCCTACAGCCATACCTCCTGCCAG 763

 976 ...............GlyValPheArgGlnGlyCysThrAlaPheArgVa 987
                     |||:::||||||||||||||||||||||||||||||
 764 AGGAAGAGCCGGGGAGGCATGTTCAGACAGGGGTGCACAGCCTTCAGGGT 813

 987 lIleThrProAsnIleAspGluGluAlaSerMetMetGluAspValGlyM 1004
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 814 CATCACACCAAACATTGATGAAGAGGCTTCCATGATGGAAGATGTCGGCA 863

1004 etGlnAspValHisPheAsnGluAspValLeuMetGluLeuLeuGluGln 1020
     ||||||||||||||||||||||||||||||||||||||||||||||||||
 864 TGCAGGATGTCCATTTCAATGAGGATGTGCTGATGGAGCTGCTGGAGCAG 913

1021 CysAlaAspGlyLeuTrpLysAlaGluArg.TyrGluLeu.IleAlaAsp 1036
     |||||||||||||||||||||||||||||| |||||||| |||||||||
 914 TGCGCAGATGGACTTTGGAAGGCAGAGCGGCTACGAGCTGGATTGCTGAC 963

1037 IleTyrLysLeuIleIleProIleTyrGluLysArgArgAsp........ 1050
     |||||||||||||||||||||||||||||||||||||
 964 ATCTATAAACTCATCATCCCCATCTATGAAAAGCGGAGGGACTTTAGAGA 1013

1050 ..................................................  1050

1014 CTACCCACCTGTATGACACCCTGCACCGCCCATACAGCAAAGTGACAGAG 1063

1050 ..................................................  1050

1064 GTCATCACTCGGGCCGCAGGCTCCTGGGACCTACTTCCGGGTGGCCTCTT 1113

1051 .........Phe.PheGluAspGluAspGlyLysGluTyrIleTyrLysG 1064
             ::: ||||||||||||||||||||||||||||||||||||||
1114 CGGACAGGGATTCTTCGAAGATGAAGACGGGAAGGAATACATCTACAAAG 1163

1064 luProLysLeuThrProLeuSerGluIleSerGlnArgLeuLeuLysLeu 1080
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1164 AGCCCAAACTCACGCCTCTGTCAGAGATTTCTCAGAGACTCCTTAAACTT 1213

1081 TyrSerAspLysPheGlySerGluAsnValLysMetIleGlnAspSerGl 1097
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1214 TACTCGGATAAATTCGGTTCTGAAAATGTCAAAATGATACAGGATTCTGG 1263

1097 yLysValAsnProLysAspLeuAspSerLysTyrAlaTyrIleGlnValT 1114
     ||||||||||||||||||||||||||||||||:::||||||||||||||||
1264 CAAGGTCAACCCGAAGGATCTGGATTCCAAGTTTGCTTACATCCAGGTGA 1313

1114 hrHisValIleProPhePheAspGluLysGluLeuGlnGluArgLysThr 1130
     ||||||||  ||||||||||||||||||||||||||||||||||||||||
1314 CCCATGTGACCCCGTTCTTTGATGAAAAGGAGTTACAGGAGAGGAAAACA 1363

1131 GluPheGluArgSerHisAsnIleArgArgPheMetPheGluMetProPh 1147
     ||||||||||||  ||||||||||||||||||||||||||||||||||||
1364 GAGTTTGAACGATGTCACAACATCCGGCGCTTCATGTTCGAGATGCCCTT 1413

1147 eThrGlnThrGlyLysArgGlnGlyGlyValGluGluGlnCysLysArgA 1164
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1414 CACCCAGACTGGGAAGAGGCAGGGTGGCGTGGAGGAGCAGTGTAAGCGAC 1463

\164 rgThrIleLeuThrAlaIleHisCysPheProTyrValLysLysArgIle 1180
     ||||||||||||||||||||||||||||||||||||||||||||||||||
   \ GGACCATCCTGACAGCAATACACTGCTTCCCCTATGTAAAGAAGCGGATC 1513

    \oValMetTyrGlnHisHisThrAspLeuAsnProIleGluValAlaIl 1197
```

```
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1514 CCTGTCATGTACCAGCACCACACTGACCTGAACCCCATTGAGGTGGCCAT 1563

1197 eAspGluMetSerLysLysValAlaGluLeuArgGlnLeuCysSerSerA 1214
     ||||||||||||||||||||||||||||||||||:::||||||||||||||
1564 CGATGAAATGAGCAAGAAAGTGGCCGAGCTCCACCAGCTGTGTTCCTCAG 1613

1214 laGluValAspMetIleLysLeuGlnLeuLysLeuGlnGlySerValSer 1230
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1614 CTGAAGTGGACATGATCAAACTGCAGCTCAAACTGCAGGGCAGTGTGAGC 1663

1231 ValGlnValAsnAlaGlyProLeuAlaTyrAlaArgAlaPheLeuAspAs 1247
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1664 GTCCAGGTCAATGCTGGCCCGCTAGCATACGCCCGAGCCTTCCTCGATGA 1713

1247 pThrAsnThrLysArgTyrProAspAsnLysValLysLeuLeuLysGluV 1264
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1714 CACCAACACAAAGAGATACCCTGACAATAAGGTGAAGCTGCTGAAGGAAG 1763

1264 alPheArgGlnPheValGluAlaCysGlyGlnAlaLeuAlaValAsnGlu 1280
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1764 TTTTCAGGCAATTCGTGGAAGCTTGTGGCCAAGCCTTGGCAGTGAACGAA 1813

1281 ArgLeuIleLysGluAspGlnLeuGluTyrGlnGluGluMetLysAlaAs 1297
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1814 CGTCTGATTAAAGAGGACCAGCTGGAGTACCAGGAAGAGATGAAAGCCAA 1863

1297 nTyrArgGluMetAlaLysGluLeuSerGluIleMetHisGluGlnIleC 1314
     ||||||||||:::   ||||||||||||:::|||:::      :::||||
1864 CTACAGGGAGATCCGGAAGGAGCTCTCCGACATCATCGTGCCGAGAATTT 1913

1314 ysProLeuGluGluLysThrSerValLeuProAsnSerLeuHisIlePhe 1330
     |||||      |||:::|||||||||||||||||||||||||||||||||
1914 GCCCTG.....GAGAGGACAAGCGTGCTACCAAATTCCCTGCACATCTTC 1958

1331 AsnAlaIleSerGlyThrProThrSerThrMetValHisGlyMetThrSe 1347
     ||||||||||||||||||||||||||||||:::|||:::|||:::||||||
1959 AACGCCATCAGCGGGACACCAACAAGCACAGTGGTTCAAGGGTTGACCAG 2008

1347 rSerSerValVal 1352
     ||||||||||||||
2009 TTCATCCTCAGTTGTG 2024

 ame  gb pr:AK056684
```

Appendix B


```
RESULT   5
RNTRG
LOCUS       RNTRG                   3227 bp    mRNA    linear   ROD 24-FEB-1999
DEFINITION  R.norvegicus trg mRNA.
ACCESSION   X68101
VERSION     X68101.1  GI:550419
KEYWORDS    trg gene.
SOURCE      Norway rat.
  ORGANISM  Rattus norvegicus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae;
            Rattus.
REFERENCE   1  (bases 1 to 3227)
  AUTHORS   Pianese,L.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-AUG-1992) L. Pianese, Dipartimento di Biologia,
            Patologia Cellulare e Molecolare, Via Pansini, Naples, ITALY
REFERENCE   2  (bases 1 to 3227)
  AUTHORS   Pianese,L., Porcellini,A., Avvedimento,V.E., D'Esposti,F.,
            Feliciello,A., Monticelli,A., Musti,A.M., Tortora,G., Varrone,S.
            and Cocozza,S.
  TITLE     A novel thyroid transcript negatively regulated by tsh
  JOURNAL   Mol. Biol. 13, 75-83 (1994)
FEATURES             Location/Qualifiers
     source          1. .3227
                     /organism="Rattus norvegicus"
                     /strain="Fischer"
                     /db_xref="taxon:10116"
                     /cell_line="FRTL-"
                     /clone_lib="lambdaGT"
     gene            1. .2218
                     /gene="trg"
     CDS             <1. .2218
                     /gene="trg"
                     /codon_start=2
                     /protein_id="CAA48220.1"
                     /db_xref="GI:550420"
                     /db_xref="SPTREMBL:Q63603"
                     /translation="KLSRGHSPLMKKVFDVYLCFLQKHQSEMALKNVFTALRSLIYKF
                     PSTFYEGRADMCASLCYEVLKCCNSKLSSIRTEASQLLYFLMRNNFDYTGKKSFVRTH
                     LQVIISLSQLIADVVGIGGTRFQQSLSIINNCANSDRLIKHTSFSSDVKDLTKRIRTV
                     LMATAQMKEHENDPEMLVDLQYSLAKSYASTPELRKTWLDSMARIHVKNGDLSEAAMC
                     YVHVTALVAEYLTRKEADLALQREPPVFPYSHTSCQRKSRGGMFRQGCTAFRVITPNI
                     DEEASMMEDVGMQDVHFNEDVLMELLEQCADGLWKAERLRAGLLTSINSSSPSMKSGG
                     TLETTHLYDTLHRPYSKVTEVITRAAGSWDLLPGGLFGQGFFEDEDGKEYIYKEPKLT
                     PLSEISQRLLKLYSDKFGSENVKMIQDSGKVNPKDLDSKFAYIQVTHVTPFFDEKELQ
                     ERKTEFERCHNIRRFMFEMPFTQTGKRQGGVEEQCKRRTILTAIHCFPYVKKRIPVMY
                     QHHTDLNPIEVAIDEMSKKVAELHQLCSSAEVDMIKLQLKLQGSVSVQVNAGPLAYAR
                     AFLDDTNTKRYPDNKVKLLKEVFRQFVEACGQALAVNERLIKEDQLEYQEEMKANYRE
                     IRKELSDIIVPRICPGEDKRATKFPAHLQRHQRDTNKHSGSRVDQFILSCVTLPHEPH
                     VGTCFVMCKLRTTFRANHWFCQAQEEAMGNGREKEPWTVIFNSRFYRSWGKVHIFF"
BASE COUNT      905 a    738 c    777 g    807 t
ORIGIN
```

```
Query Match          28.1%;  Score 1349.8;  DB 10;  Length 3227;
Best Local Similarity   78.4%;  Pred. No. 0;
Matches 1838;  Conservative    0;  Mismatches  297;  Indels  210;  Gaps   10;

Qy   2237 catggacataatcctctcatgaaaaaagtttttgatgtctacctgtgttttcttcaaaaa 2296
          | |||||||| ||| |||||||| ||||||||||||||||||||||||||||| || |||||
Db     11 CGTGGACATAGTCCACTCATGAAGAAAGTTTTTGATGTCTACCTGTGTTTCCTCCAAAAG 70

Qy   2297 catcagtctgaaacggctttaaaaaatgtcttcactgccttaaggtccttaatttataag 2356
          |||||||| |||| |||||||||||||||||||||||||||||||||||| ||||||||||||
Db     71 CATCAGTCAGAAATGGCTTTAAAAAATGTCTTCACTGCCTTAAGGTCATTAATTTATAAG 130

Qy   2357 tttccctcaacattctatgaagggagagcggacatgtgtgcggctctgtgttacgagatt 2416
          || |||||||| ||||| || ||| | || |||||||| ||  ||||||| || ||| ||
Db    131 TTCCCCTCAACGTTCTACGAGGGGCGGGCAGACATGTGCGCATCTCTGTGCTATGAGGTT 190

Qy   2417 ctcaagtgctgtaactccaagctgagctccatcaggacggaggcctcccagctgctctac 2476
          |||||||||||||||||||||||||||| |||||||| | || ||||||||||||||||||||
Db    191 CTCAAGTGCTGTAACTCCAAGCTCAGCTCCATCCGCACCGAGGCCTCCCAGCTGCTCTAC 250

Qy   2477 ttcctgatgaggaacaactttgattacactggaaagaagtcctttgtccggacacatttg 2536
          |||||||||||||||||||| || ||||| || |||||||| ||||||||||||| ||
Db    251 TTCCTGATGAGGAACAACTTCGACTACACAGGGAAGAAGTCTTTTGTCCGGACACACTTA 310

Qy   2537 caagtcatcatatctgtcagccagctgatagcagacgttgttggcattggggaaaccaga 2596
          || |||||||| ||| |||||||||||| |||||||| |||||||||| | |||||||
Db    311 CAGGTCATCATCTCTCTCAGCCAGCTGATCGCAGACGTGGTTGGCATTGGAGGAACCAGA 370

Qy   2597 ttccagcagtccctgtccatcatcaacaactgtgccaacagtgaccggcttattaagcac 2656
          |||||||||||| |||| |||||||||||||||||| || |||||||| |||||||||
Db    371 TTCCAGCAGTCCTTGTCTATCATCAACAACTGTGCCAATAGCGACCGGCTCATTAAGCAC 430

Qy   2657 accagcttctcctctgatgtgaaggacttaaccaaaaggatacgcacggtgctaatggcc 2716
          |||||||||||||||||||||||||||||||| || ||||| ||||| |||| || ||||||
Db    431 ACCAGCTTCTCCTCTGATGTGAAGGATTTGACCAAGAGGATCCGCACAGTCCTGATGGCC 490

Qy   2717 accgcccagatgaaggagcatgagaacgacccagagatgctggtggacctccagtacagc 2776
          || |||||||||||||| |||||||||||||||||||||||||||||||||| |||||||||
Db    491 ACAGCCCAGATGAAGGAACATGAGAACGACCCAGAGATGCTGGTGGACCTGCAGTACAGC 550

Qy   2777 ctggccaaatcctatgccagcacgcccgagctcaggaagacgtggctcgacagcatggcc 2836
          |||||||| |||| |||||||| || |||||||||||||| |||| ||||||||||||
Db    551 CTGGCCAAGTCCTACGCCAGCACCCCTGAGCTCAGGAAGACATGGCTGGACAGCATGGCA 610

Qy   2837 aggatccatgtcaaaaatggcgatctctcagaggcagcaatgtgctatgtccacgtaaca 2896
          |||||||||| |||||||| |||||||||||||||||||||||||||||||||||| |||
Db    611 AGGATCCATGTTAAAAATGGGGATCTCTCAGAGGCAGCAATGTGCTATGTCCACGTGACA 670
```

Appendix B (cont.)

```
Qy   2897 gccctagtggcagaatatctcacacggaa---------------------------- 2925
          ||  | || || ||||||||||||||||
Db    671 GCTTTGGTAGCGGAATATCTCACACGGAAAGAAGCTGACCTAGCACTCCAGCGGGAACCA 730

Qy   2926 ---------------------------------------------aggcgtgtttaga 2938
                                                       |||| |||| |||
Db    731 CCTGTCTTCCCCTACAGCCATACCTCCTGCCAGAGGAAGAGCCGGGGAGGCATGTTCAGA 790

Qy   2939 caaggatgcaccgccttcagggtcattaccccaaacatcgacgaggaggcctccatgatg 2998
          || || ||||| |||||||||||||| || |||||||| || || ||||| |||||||||
Db    791 CAGGGGTGCACAGCCTTCAGGGTCATCACACCAAACATTGATGAAGAGGCTTCCATGATG 850

Qy   2999 gaagacgtggggatgcaggatgtccatttcaacgaggatgtgctgatggagctccttgag 3058
          ||||| || || |||||||||||||||||||| |||||||||||||||||||| || |||
Db    851 GAAGATGTCGGCATGCAGGATGTCCATTTCAATGAGGATGTGCTGATGGAGCTGCTGGAG 910

Qy   3059 cagtgcgcagatggactctggaaagccgagc-gctacgagct-catcgccgacatctaca 3116
          |||||||||||||||| ||||| || |||| |||||||||| || || |||||||| |
Db    911 CAGTGCGCAGATGGACTTTGGAAGGCAGAGCGGCTACGAGCTGGATTGCTGACATCTATA 970

Qy   3117 aacttatcatccccatttatgagaagcggaggga---------------------------- 3150
          |||| |||||||||| ||||| ||||||||||
Db    971 AACTCATCATCCCCATCTATGAAAAGCGGAGGGACTTTAGAGACTACCCACCTGTATGAC 1030

Qy   3151 ---------------------------------------------------------- 3150

Db   1031 ACCCTGCACCGCCCATACAGCAAAGTGACAGAGGTCATCACTCGGGCCGCAGGCTCCTGG 1090

Qy   3151 -------------------------------tttctttgaagatgaagatggaaaggag 3178
                                            ||||| |||||||||| || |||||
Db   1091 GACCTACTTCCGGGTGGCCTCTTCGGACAGGGATTCTTCGAAGATGAAGACGGGAAGGAA 1150

Qy   3179 tatatttacaaggaacccaaactcacaccgctgtcggaaatttctcagagactccttaaa 3238
          || || ||||| || |||||||||| || |||||| || ||||||||||||||||||||
Db   1151 TACATCTACAAAGAGCCCAAACTCACGCCTCTGTCAGAGATTTCTCAGAGACTCCTTAAA 1210

Qy   3239 ctgtactcggataaatttggttctgaaaatgtcaaaatgatacaggattctggcaaggtc 3298
          || |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Db   1211 CTTTACTCGGATAAATTCGGTTCTGAAAATGTCAAAATGATACAGGATTCTGGCAAGGTC 1270

Qy   3299 aaccctaaggatctggattctaagtatgcatacatccaggtgactcacgtcatccccttc 3358
          ||||| |||||||||||||| |||| ||| |||||||||||||| || || | ||| |||
Db   1271 AACCCGAAGGATCTGGATTCCAAGTTTGCTTACATCCAGGTGACCCATGTGACCCCGTTC 1330

Qy   3359 tttgacgaaaaagagttgcaagaaaggaaaacagagtttgagagatcccacaacatccgc 3418
          ||||| ||||| ||||| || || |||||||||||||||||  |||  ||||||||||
Db   1331 TTTGATGAAAAGGAGTTACAGGAGAGGAAAACAGAGTTTGAACGATGTCACAACATCCGG 1390

Qy   3419 cgcttcatgtttgagatgccatttacgcagaccgggaagaggcagggcggggtggaagag 3478
          |||||||||| |||||||| || || ||||| |||||||||||||| || ||||| |||
Db   1391 CGCTTCATGTTCGAGATGCCCTTCACCCAGACTGGGAAGAGGCAGGGTGGCGTGGAGGAG 1450

Qy   3479 cagtgcaaacggcgcaccatcctgacagccatacactgcttcccttatgtgaagaagcgc 3538
          ||||| || || || |||||||||||||| |||||||||||||| ||||| ||||||||
Db   1451 CAGTGTAAGCGACGGACCATCCTGACAGCAATACACTGCTTCCCCTATGTAAAGAAGCGG 1510

Qy   3539 atccctgtcatgtaccagcaccacactgacctgaaccccatcgaggtggccattgacgag 3598
          |||||||||||||||||||||||||||||||||||||||||||||| |||||||||| ||
Db   1511 ATCCCTGTCATGTACCAGCACCACACTGACCTGAACCCCATTGAGGTGGCCATCGATGAA 1570

Qy   3599 atgagtaagaaggtggcggagctccggcagctgtgctcctcggccgaggtggacatgatc 3658
          ||||| ||||| ||||| |||||||  |||||||| ||||| || || ||||||||||||
Db   1571 ATGAGCAAGAAAGTGGCCGAGCTCCACCAGCTGTGTTCCTCAGCTGAAGTGGACATGATC 1630

Qy   3659 aaactgcagctcaaactccagggcagcgtgagtgttcaggtcaatgctggcccactagca 3718
          |||||||||||||||| |||||||| ||||| || ||||||||||||||| ||||||
Db   1631 AAACTGCAGCTCAAACTGCAGGGCAGTGTGAGCGTCCAGGTCAATGCTGGCCCGCTAGCA 1690

Qy   3719 tatgcgcgagctttcttagatgatacaaacacaaagcgatatcctgacaataaagtgaag 3778
          || || ||||| ||| | ||||| || ||||||||| |||| |||||||||| ||||||
Db   1691 TACGCCCGAGCCTTCCTCGATGACACCAACACAAAGAGATACCCTGACAATAAGGTGAAG 1750
```

```
Qy   3779 ctgcttaaggaagttttcaggcaatttgtggaagcttgcggtcaagccttagcggtaaac 3838
          ||||| |||||||||||||||||||| |||||||||| || |||||||| || || |||
Db   1751 CTGCTGAAGGAAGTTTTCAGGCAATTCGTGGAAGCTTGTGGCCAAGCCTTGGCAGTGAAC 1810

Qy   3839 gaacgtctgattaaagaagaccagctcgagtatcaggaagaaatgaaagccaactacagg 3898
          |||||||||||||||| |||||||| ||||| ||||||| ||||||||||||||||||
Db   1811 GAACGTCTGATTAAAGAGGACCAGCTGGAGTACCAGGAAGAGATGAAAGCCAACTACAGG 1870

Qy   3899 gaaatggcgaaggagctttctgaaatcatgcatgagcagatctgccccctggaggagaag 3958
          || ||   ||||||||| || || |||||      |   || |||||      ||||| |
Db   1871 GAGATCCGGAAGGAGCTCTCCGACATCATCGTGCCGAGAATTTGCCC-----TGGAGAGG 1925

Qy   3959 acgagcgtcttaccgaattcccttcacatcttcaacgccatcagtgggactccaacaagc 4018
          || |||||  |||| |||||||| |||||||||||||||||||| ||||| |||||||||
Db   1926 ACAAGCGTGCTACCAAATTCCCTGCACATCTTCAACGCCATCAGCGGGACACCAACAAGC 1985

Qy   4019 acaatggttcacgggatgaccagctcgtcttcggtcgtgtgattacatctcatggcccgt 4078
          ||| ||||||| ||| |||||||| || || || || |||||| |  | ||||||  ||
Db   1986 ACAGTGGTTCAAGGGTTGACCAGTTCATCCTCAGTTGTGTGACTTTACCTCATGAACCAC 2045

Qy   4079 gtgtggggacttgctttgtcatttgcaaactcaggatgctttccaaagccaatcactggg 4138
            |||||||| |||||||||| |||||||||||| |  || || ||| |||||||||
Db   2046 ACGTGGGGACATGCTTTGTCATGTGCAAACTCAGGACGACTTTCAGAGCTAATCACTGGT 2105

Qy   4139 gagaccgagcacagggagga-ccaaggggaaggggagagaaaggaaataaagaacaacgt 4197
              || |||||||| || | ||| |||||| |||||||| |   |     | ||   |
Db   2106 TTTGCCAAGCACAGGAAGAAGCCATGGGGAATGGGAGAGAGAAAGAGCCCTGGACTGTGA 2165

Qy   4198 tatttcttaacagactttctataggagttgtaagaaggtgcacatatttttttaaatctc 4257
          |||||  || ||||  || ||||||||||   |||||||||||||||||||||||||||
Db   2166 TATTTAATAGCAGA--TTTTATAGGAGTTGGGGGAAGGTGCACATATTTTTTTAAATCTC 2223

Qy   4258 actggcaatattcaaagttttcattgtgtcttaacaaaggtgtggtagacactcttgagc 4317
          ||||||||| ||   ||||||| |  |||||||||   || | || || ||||||| ||
Db   2224 ACTGGCAATGTT--TAGTTTTCCTCATGTCTTAACAGGTGTAT-GTGGATACTCTTGGGC 2280

Qy   4318 tggacttagattttattcttccttgcagagtagtgttagaatagatggcctacagaaaaa 4377
          || | ||  |||||||||||| ||| |||  |||| ||| |||| |||| ||| || ||||
Db   2281 TGCAGTTCAATTTTATTCTT-CTTACAGTATAGTATTAAAATAAATGGTCTAAAGGAAAA 2339

Qy   4378 aaagg 4382
            | ||
Db   2340 GAGGG 2344
```

RESULT 6
BC0[illegible]